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A new type of GC–MS with advanced capabilities

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Abstract

We have combined the benefits of supersonic molecular beam interface and its related fly-through electron ionization (EI) ion source with the advanced features of the Varian 1200L gas chromatography–mass spectrometry (GC–MS) and mass spectrometry–mass spectrometry (MS–MS), resulting in a new and powerful GC–MS platform with record setting performance. Electron ionization of vibrationally cold molecules in the supersonic molecular beams (SMB) (cold EI) provided mass spectra with enhanced molecular ion, yet with good library search results and superior identification probabilities. We found that high GC column flow rates lower the elution temperature for any given compounds. This allows much larger molecules to elute at the maximum temperature of standard columns. We analyzed a mixture of heavy linear chain hydrocarbons all the way to $C_{84}H_{170}$ with a molecular weight of 1179.3 amu, using a 4 m 0.25 mm i.d. column and 32 ml/min He flow rate. Furthermore, we obtained a dominant molecular ion to all these compounds. The lower elution temperatures also greatly enhance the ability to analyze very thermally labile compounds such as carbamate pesticides. The experimental 1200 system is capable of triple quadrupole based MS–MS. We found that MS–MS on the molecular ion is much more effective than on fragment ions, and thus, the enhancement of the molecular ion directly improves the MS–MS sensitivity. Fast GC–MS analysis was also explored, based on very high column flow rate for fast splitless injections without affecting the sensitivity, and on the high system selectivity due to the combination of enhanced molecular ion and MS–MS. We demonstrate a few seconds long GC–MS–MS analysis of diazinon, spiked at 10 ng/g in a mixed fruit and vegetable extract. The feature of enhanced molecular ion provides significant enhancement in the detection sensitivity via SIM and RSIM on the molecular ion. While octafluoronaphthalene (OFN) detection limit of below 1 fg in SIM mode is shown, the harder is the compound analysis by standard GC–MS the greater is the gain in sensitivity by 1200-SMB. For example, the use of the 1200-SMB resulted in an improvement of over 50 times in the RSIM detection limit of cholesterol on its molecular ion at $m/z = 386$, and decabromobiphenylether could be analyzed in SIM mode at 1 ppb with S/N > 50 (peak-to-peak noise).

Keywords: Supersonic molecular beams; GC–MS; MS–MS; Fast GC–MS; Enhanced molecular ion

1. Introduction

Gas chromatography–mass spectrometry (GC–MS) is a central analytical technique that serves in a broad range of applications. However, GC–MS suffers from a major "Achilles Heel" in the form of a relatively small range of volatile, thermally stable compounds that are amenable for analysis. This limited capability with respect to analyzing thermally labile and low volatility large molecules significantly restricts the usefulness and growth potential of GC–MS.

The major strength of GC–MS using electron ionization (EI) is its ability in compound identification through the use of extensive and established 70 eV EI libraries. However, EI mass spectra suffer from frequent absence of the molecular ion, which otherwise can be weak and results in reduced confidence level in the sample identification.

In addition, the observation of the molecular ion is sometimes essential for the verification of the identity of the measured compound since the mass spectra of its degradation products often have similar fragmentation patterns but different molecular ion. Chemical ionization (CI) could be used for the provision of molecular ions; however, it is ineffective with several important classes of compounds such as aliphatics, usually much less sensitive than EI and incompatible with library search and identification. Furthermore, in some instruments it requires hardware changes.

We note that the above mentioned problems of weak or missing molecular ion and the unsatisfied (by GC–MS) yet ever

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growing need in the analysis of thermally labile and larger size compounds are linked together since these compounds are typically characterized by having weak or no molecular ions.

In order to address these growing needs we have developed and explored the performance capabilities of a unique type of GC–MS, based on the use of supersonic molecular beams (SMB) for interfacing the GC to the MS [1–9] and as a medium for the ionization of sample compounds while in the SMB, either by electron ionization [2,6,10,11] or by hyperthermal surface ionization (HSI) [10,12].

Supersonic molecular beams are characterized by intramolecular vibrational supercooling, unidirectional motion with controlled hyperthermal kinetic energy (1–20 eV), mass focusing similar to that in a jet separator and capability to handle very high column flow rates of up to 240 ml/min [1,6]. While our research has employed a quadrupole mass analyzer [1–11], the same as Kishi et al. [13], GC–MS with supersonic molecular beams was also implemented with a time-of-flight mass analyzer [14,15]. GC–MS with supersonic molecular beams was also explored in combination with laser based ionization methods [16–18].

GC–MS with SMB was initially explored by us using a laboratory-made instrument [1–5], and later on it was combined with an Agilent model 6890 GC plus 5972 MSD [6–9] and it was named "Supersonic GC–MS".

In order to better address the growing challenges and further advance the Supersonic GC–MS, we recently combined the benefits of supersonic molecular beam interface and its related fly-through EI ion source with the advanced features of the Varian 1200L GC–MS and MS–MS, resulting in a new and powerful GC–MS platform with record setting performance. In comparison with the previously published Supersonic GC–MS [6] the new one that is based on the Varian 1200L GC–MS system (named 1200 Supersonic GC–MS or 1200-SMB) has: (a) extended mass range of 1500 amu in comparison with 700 amu of the 5972 MSD; (b) MS–MS capability; (c) all the vacuum chambers including that of the nozzle are pumped by turbo molecular pumps hence cluster chemical ionization [19,20] is enabled and (d) neutral based mass independent noise is eliminated through the use of 180° curved Q_2, thus in combination with the elimination of vacuum background lower noise is obtained hence lower detection limits can be achieved.

2. The "Supersonic GC–MS" system

The principles of GC–MS with supersonic molecular beams were described in detail in Refs. [1,6,10]. Therefore, in this section the description will focus on its modifications and new features as employed in the design of the new system. Fig. 1 shows a schematic diagram of the 1200-SMB, and it is mostly self explained with the names of all its major components. As the name implies it is based on the Varian 1200L GC–MS system (Varian, Walnut Creek, CA, USA). The bottom schematic diagram is a zoom on the modified portion of the SMB interface, fly-through EI ion source and its 90° ion mirror.

The column output is mixed with ~90 ml/min helium make up gas and flow through a heated (and temperature controlled) transfer line to the supersonic nozzle. The helium make up gas can be mixed via the opening of a valve with perfluorotributylamine (PFTBA) which serves for the system tuning and calibration. The helium make up gas can also be mixed with methanol vapor for inducing cluster chemical ionization [19,20]. A second column is also connected from a second 1079 PTV injector (not shown) having a ChromatoProbe sample introduction device [21]. The ChromatoProbe can serve as a Probe for the investigation of cold EI of chosen compounds and for high mass tuning with perfluoroeicosane ($C_{20}F_{42}$) on its $m/z = 1018$ high mass fragment or $m/z = 1038$ molecular ion. The ChromatoProbe can also serve for the analysis of dirty samples when installed in front of a standard column [21].

The sample compounds seeded in helium make up gas expand from a 90 μm diameter supersonic nozzle into a nozzle vacuum chamber that is differentially pumped by a Varian Navigator 301 turbo molecular pump (Varian Inc., Torino, Italy) having 250 l/s pumping speed. The helium pressure at this vacuum chamber is about 6 μbar.

The supersonic expansion vibrationally cools the sample compounds and the expanded supersonic free jet is skimmed by a 0.8 mm skimmer and collimated in a second differentially pumped vacuum chamber into a supersonic molecular beam. The second vacuum chamber is pumped by a second Varian Inc. 300/400 split turbo molecular pump that pumps both the second vacuum chamber (400 l/s) and main mass spectrometer vacuum chamber (300 l/s). The supersonic molecular beam with seeded vibrationally cold sample compounds fly-through a dual cage electron ionization ion source [22] where it is ionized by 70 eV electrons with 10 mA emission current. The ions are focused by ion lens system, deflected 90° by an ion mirror and enter an RF only quadrupole ion transfer optics Q_0 of the original 1200 system. The 90° ion mirror serves to keep the mass analyzers clean from sample induced contaminations and it is separately heated. The ions are further transferred through an ion lens into the mass spectrometer vacuum chamber and are analyzed by a system of three quadrupole mass analyzers Q_1, Q_2 and Q_3, where each can be operated in the RF ion transfer mode while Q_1 and Q_3 can be operated in either single ion monitoring or full scan modes as commonly employed in MS–MS based triple quadrupole MS systems. Since Q_2 is a 180° curved RF only quadrupole ion transfer system, a head on ion detector is positioned directly at the ion path of ions exiting Q_3 and it is biased on 5 kV while serving also as an efficient ion to electron converter. Due to the 180° turning of Q_2 the so-called mass independent neutral noise was lower than one count in 10 s.

Several new design features were used in this 1200 Supersonic GC–MS in comparison with the previously described Supersonic GC–MS [6], including:

1. A commercially available Varian 1200L GC–MS was used as the basic platform without any irreversible changes. The major design guideline was that the supersonic molecular beam interface and its associated fly-through EI ion source and ion optics will be an add-on to the standard 1200L system.

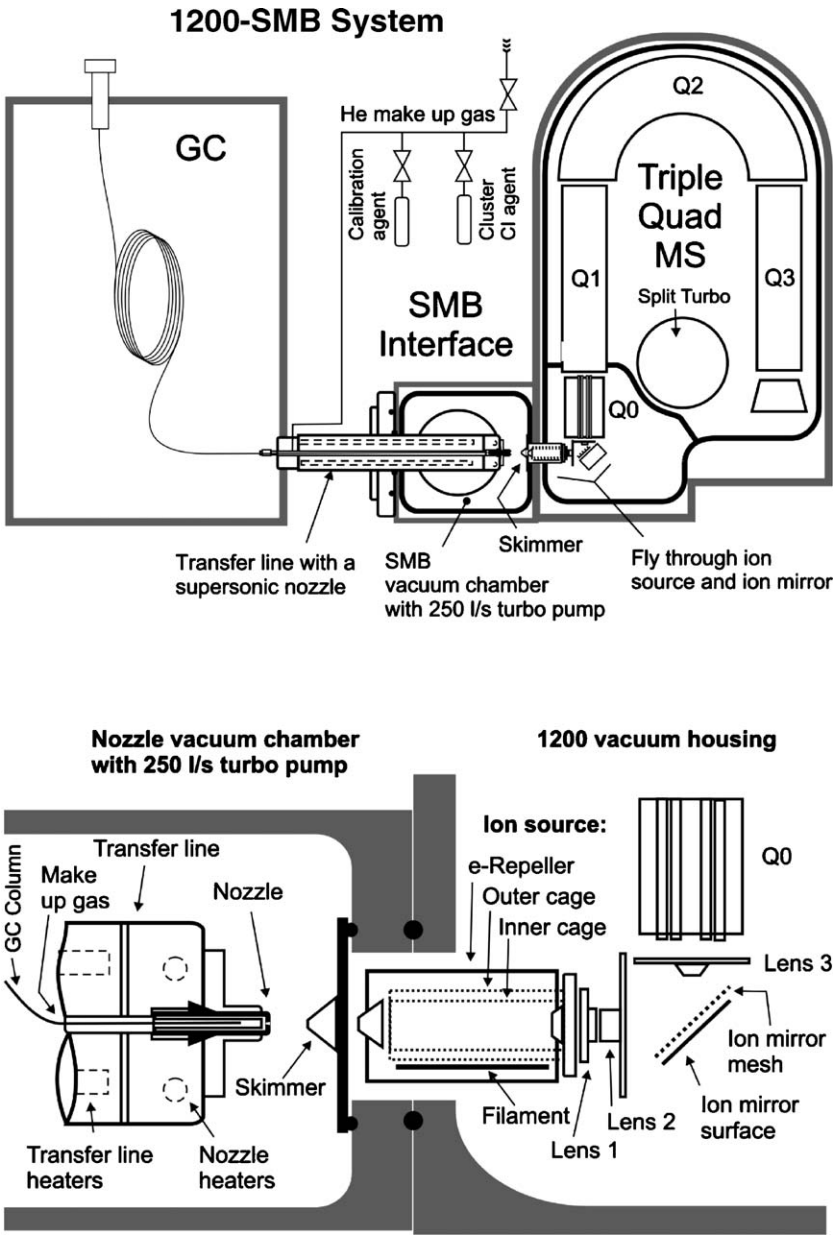

Fig. 1. A schematic diagram of the 1200-SMB system. The various components are drawn approximately to scale and main component names are included. Further explanations are given in the text.

2. Helium make up gas was provided by a standard needle valve and similarly the PFTBA mass calibration compound and methanol cluster CI vapor are controlled by a manual on–off valve. In the near future the manual valves will be replaced by an auxiliary electronic pressure controlled (EPC) solenoid valves.
3. Two GC columns were simultaneously connected via a dual-hole ferrule into the transfer line. One column was connected to a 1177 split–splitless or 1079 PTV injector and the second column was connected from a 1079 PTV injector having a ChromatoProbe [21] with a short (1 m) microbore (0.1 mm i.d.) transfer line.
4. The transfer line has two separately controlled heated zones: (a) the supersonic nozzle and (b) the main body that was capable of temperature programming for the further optimization of the analysis of thermally labile compounds. The transfer line and nozzle could be heated to over 400 °C. Forced air cooling of the transfer line was explored, and we measured its cooling rate as 23 °C/min at 200 °C.
5. The nozzle was made from Vespel, with nozzle diameter of 90 μm and it has an internal fused silica tubing liner where the columns ends and column flow is mixed with make up flow.
6. The transfer line and nozzle structure could be aligned in front of a skimmer (0.8 mm diameter) without opening the vacuum system with an XYZ motion manipulation system.
7. The original Varian model 102 rotary pump (100 l/min) (Varian Inc.) served for the parallel pumping of both turbo molecular pumps.

8. The dual cage fly-through EI ion source is mounted on a flat alumina ceramic piece and it is located in the standard transfer line left side vacuum port. This fly-through ion source operates with about 10 mA electron emission current from a thoriated-tungsten ribbon filament (30 mm long, 0.75 mm wide and 0.025 mm thick) and its inner ion cage diameter is 10 mm.
9. The original EI ion source was removed and was not used. The front located original ion volume replacement device was removed and replaced by a vacuum feedthrough for the fly-through EI ion source and its ion optics.
10. A 90° ion mirror element was located after the fly-through EI ion source. It could be combined with a directly heated rhenium HSI surface [10,12] but in all the reported experiments a 50 μm thick directly heated Hastalloy 625 foil was used for ion deflection, positioned 1.5 mm behind an open mesh front. The mesh was made from 25 μm gold coated tungsten wires with 50 units per inch. This ion mirror element was positioned at 45° to both the SMB axis and the axis of the Q_0 and Q_1 mass analyzer.
11. The added bench space required by the addition of the supersonic nozzle differential pumping vacuum chambers and its heated transfer line was 22 cm.
12. Ten external power supplies were used in the experiments described in this manuscript. A computer controlled mass-programmed dedicated electronics is under final stages of construction.

3. Results and performance evaluation

3.1. Cold EI, cool classical EI and library search

The basic feature of the 1200-SMB is its ability to provide electron ionization mass spectra of vibrationally cold sample compounds in the supersonic molecular beams. These mass spectra are named "cold EI" and are characterized by enhanced molecular ion and isomer mass spectral effects. Cold EI mass spectrometry of aliphatic compounds, pesticides and many other compounds in supersonic molecular beams has been extensively studied in the past [2,7,10]. However, since the 1200-SMB is a new system, cold EI performance should be demonstrated with it and the results should be compared with those obtained with other systems.

In Fig. 2, the 70 eV cold EI mass spectrum of hexadecane (*n*-$C_{16}H_{34}$) is shown (upper trace) together with the NIST library mass spectrum (bottom trace) of this compound. The cold EI mass spectrum clearly demonstrates enhanced molecular ion which shows over 30 times increased relative abundance compared with the $m/z = 57$, the most abundant fragment. Note that the fragmentation pattern is retained and it is very similar to that of the library, thus, the most prominent change in cold EI versus standard EI mass spectra is the enhancement of the molecular ion. The reason for this observation is that due to the vibrational cooling the molecular ion dissociates to a much smaller extent while all other fragment ions are in a steady state of abundances as they gain population from lower mass ions and lose population to higher mass fragments. Only the very low mass fragments

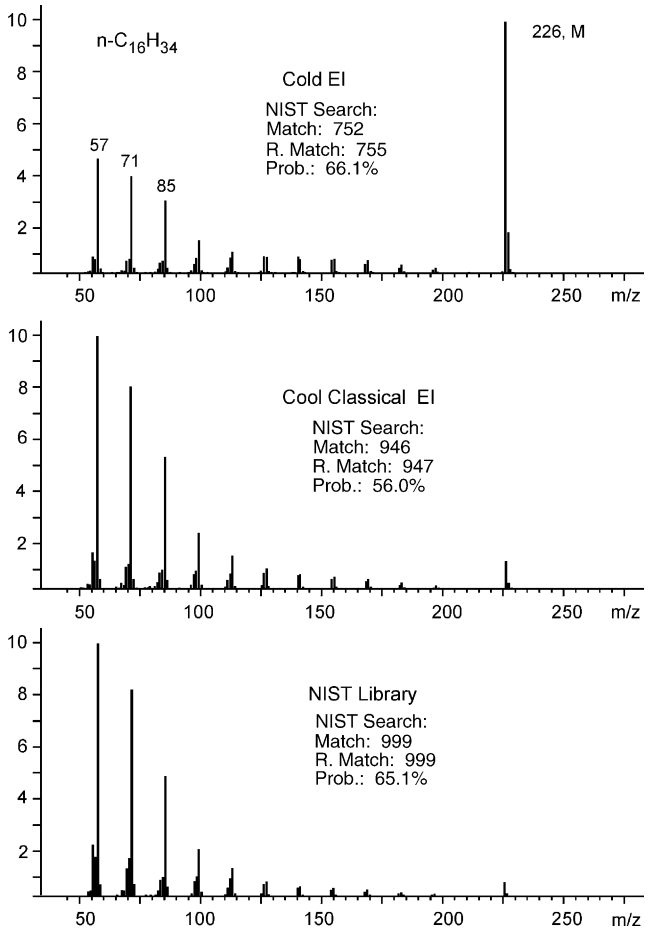

Fig. 2. Cold electron ionization mass spectrometry. Cold EI mass spectrum of hexadecane (upper trace) is compared with the classical EI-SMB (also called "cool classical EI") mass spectrum of that compound (middle trace) and the NIST 70 eV library mass spectrum of that compound. Hexadecane was continuously fed into the molecular beam from a ChromatoProbe at 150 °C 1079 injector temperature with split ratio 10. Cold EI MS was obtained with 85 ml/min He make up gas flow rate while cool classical EI was obtained with 5 ml/min He flow rate.

lose population in cold EI but their importance is minimal in any case. Note that the library has a few replica mass spectra and the enhancement of the molecular ion can amount up to 50 times depending on the library MS used for comparison.

While cold EI is characterized by enhanced molecular ion, one would like to have easy switching to classical EI mass spectra for certain applications. The 1200-SMB enables this switching simply by the reduction of the helium make up gas flow rate from 90 to 5 ml/min (two ion lens voltages are also changed a little) which can be automated in the method (can be done on a run to run basis or even within a run). Under these conditions, the vibrational cooling is ineffective while the jet separation efficiency is still good, thus the obtained EI mass spectrum is of sample molecules at the vibrational temperature of the supersonic nozzle which is similar to that of standard ion sources. As demonstrated in the middle trace of Fig. 2 our classical EI mass spectrum is very close to that of the library. Since we can control the sample vibrational temperature we can also call this mode of ionization "cool classical EI", although

a better name could be "classical EI-SMB". The classical EI-SMB mass spectrum of hexadecane is characterized by a high NIST search matching factor of 946 and reversed matching factor of 947. More important is the NIST identification probability which is 56.0%. For aliphatic compounds such as hexadecane this probability is very high. To demonstrate this fact one can take the mass spectrum from the NIST library itself and asked the NIST library to search it, as we did. Obviously, we got the highest possible matching factor of 999 but the probability was only 65.1%. The reason for this is that the library has many other aliphatic compounds, all showing the same fragmentation pattern and are different only in the molecular ion peaks which may or may not exist. We note that while cold EI gave a lower matching factor of 752, its identification probability was 66.1% which is higher than that of a perfect match of the library mass spectrum searched by the library. The clear reason for this is that the only unique mass spectral feature of the searched compound was significantly enhanced. This observation of "lower fit but better hit" of cold EI is not new and it was found also for a series of 13 pesticides [7] although for those pesticides the degree of enhancement of the molecular ion was lower.

It should be mentioned that small molecules, and those which exhibit a dominant molecular ion in conventional 70 eV EI, show "cold EI" mass spectra which are practically identical to those of thermal EI such as those in the NIST library. Examples of this "no effect" category are small aromatic compounds including benzene and hexachlorobenzene and even octafluoronaphthalene. Here, a "small" molecule means a molecule with less than about 15 atoms and without many low-frequency vibrations (low vibrational heat capacity). Medium size compounds, such as most of the pesticides, show a limited magnitude of molecular ion enhancement and thus exhibit high matching factors with library searches. In addition, these compounds usually show a lower relative abundance of the molecular ion in standard GC–MS systems in comparison with the NIST library mass spectra, since the ion source temperatures are usually higher under practical GC–MS analysis conditions than the temperatures used for obtaining the library mass spectra. Only large compounds and those with weak or no molecular ions such as large aliphatic compounds show significant enhancement in the relative abundance of the molecular ion. Finally, we note that the "cold EI" mass spectra can be added to the library for any target compound analysis.

3.2. The analysis of thermally labile carbamate pesticides

Since GC–MS suffers from a major limitation in the form of a relatively small range of volatile, thermally stable compounds that can be analyzed, the new 1200-SMB system is aimed at alleviating this Achilles Heel of GC–MS and significantly extend the range of compounds amenable for GC–MS analysis. The analysis of thermally labile and low volatility compounds was investigated with GC–MS with SMB from the first time that such combination was explored [1,3]. In these two publications the significantly reduced time of analysis was invoked as the major reason for the ability to analyze thermally labile and low volatility compounds. However, recently we revisited this subject and in a detailed study found a new insight into the parameters that affect the extension of range of compounds amenable for analysis [8]. Briefly, in order to analyze thermally labile and low volatility compounds one need to solve elution and degradation problems in the injector, column and ion source. In the 1200-SMB ion source degradation is inherently eliminated due to its fly-through nature. We found that the GC column elution temperature is affected by the column length, carrier gas flow rate, column film thickness and temperature programming rate. The use of a shorter column, higher column flow rate, thinner column film and slower temperature programming resulted in lower elution temperatures. Pyrene, which eluted at 287 °C with standard 30 m column with 1 μm DB5 film with 1 ml/min He flow rate could be eluted at 79 °C with a 1 m column with 0.1 μm DB5 film with 100 ml/min He flow rate (7 °C/min temperature programming rate). This is a significant elution temperature reduction of 209 °C, which is greater than that found in supercritical fluid chromatography (SFC) analysis of pyrene [23] in which pyrene was eluted in more than 1 h at a higher temperature of 100 °C in SFC–MS [23]. We found a general rule of ∼20 °C elution temperature reductions per each factor of two shorter column, higher flow rate, thinner column film and slower temperature programming rate.

Among these parameters, the use of high column flow rate was found as the key parameter for the analysis of thermally labile compounds. While high column flow rate by itself reduces the elution temperature, its magnitude can be programmed (unlike with column length reduction), thus it extends the range of compounds that can be analyzed. In addition, the use of high column flow rate increases the column capacity through its proportional increase of the plate height. Thus, high column flow rate enables the use of thinner films without any penalty of reduced column capacity thereby further lowering the elution temperatures. However, the most important effect of high column flow rate is that the same way as it lowers the column elution temperature it lowers the elution temperature from the injector liner to the column. Furthermore, it also enables the use of a temperature programmable injector (PTV) with a reasonably fast temperature programming rate so that each compound is transferred to the column within a narrow range of relatively low injector temperatures.

We note that while injector degradation can also be suppressed by cold on-column injections, such mode of injection cannot be used in combination with complex matrices since the column lifetime will be significantly reduced this way.

Thus, the use of high column flow rate and shorter column in combination with the fly-through cold EI ion source provided a complete solution that addressed all the parameters that affect the analysis of thermally labile and low volatility compounds [8]. The only penalty in this approach is that every 40 °C lower elution temperature due to shorter column or higher column flow rate is involved with a factor of 4 lower number of separation plates which is a factor of 2 lower GC separation resolution, while the reduction of elution temperatures with thinner films comes "free" in view of increased column capacity with high column flow rates.

In Fig. 3, the analysis of carbamate pesticide mixture including the thermally labile aldicarb, methomyl, aldicarbsulfone and oxamyl is demonstrated at the upper chromatogram. However, such analysis requires a mass spectrometric proof of sample integrity which is shown at the bottom mass spectra of aldicarb (left) and oxamyl (right) that demonstrate a molecular ion in combination with the library searchable mass spectra. In addition, cluster chemical ionization was also used [20] for the unambiguous confirmation of the identification of the molecular ion of these thermally labile carbamate pesticides.

3.3. The analysis of low volatility large hydrocarbons

Cold EI mass spectrometry and GC–MS of hydrocarbons was investigated with the Supersonic GC–MS [2,6] but due to upper mass range of 700 amu it was limited to $C_{40}H_{82}$. Since

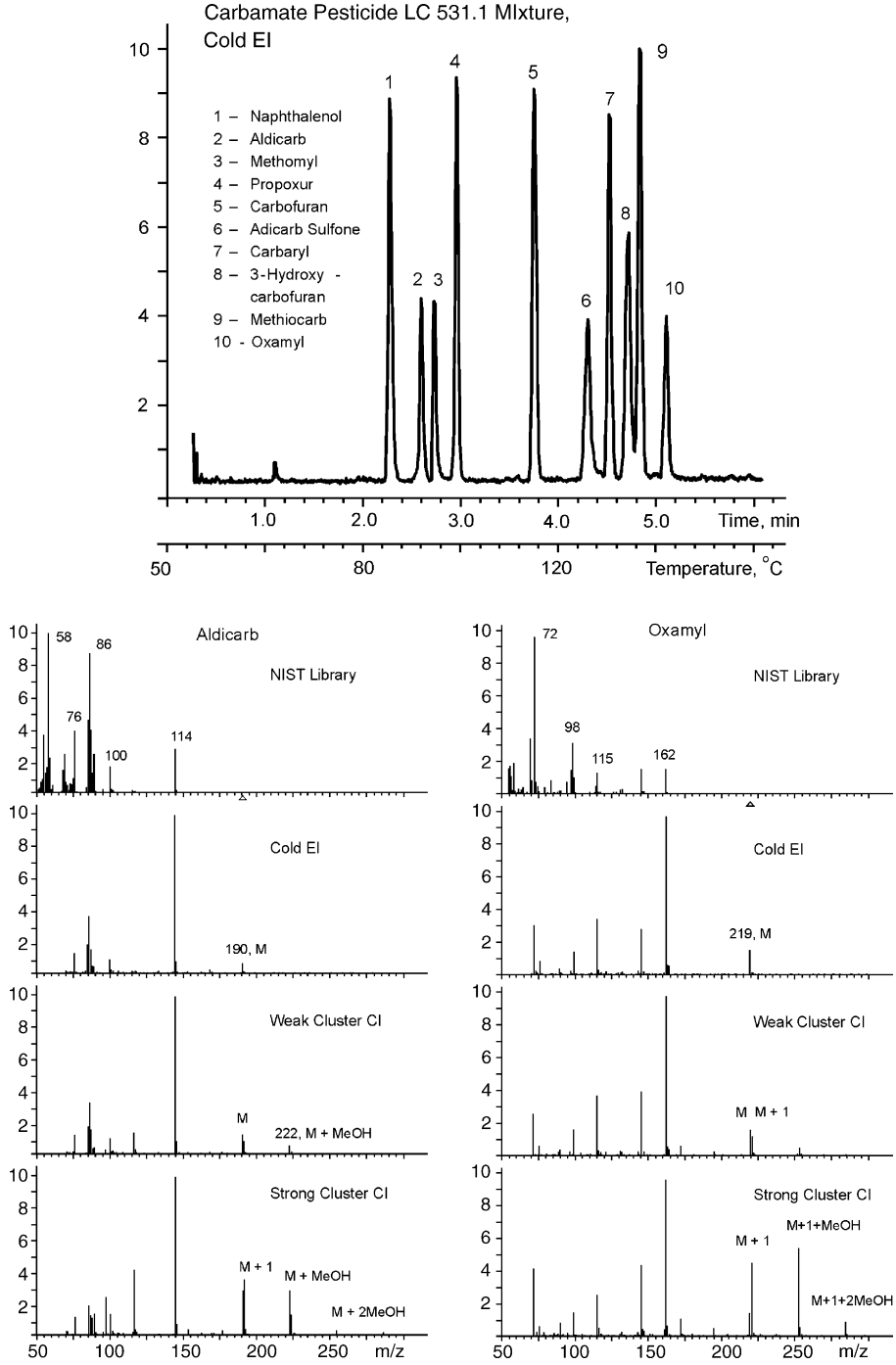

Fig. 3. The analysis of carbamate mixture with the 1200-SMB system. The pesticide names are listed in order of their elution time. Four meter column with 0.1 μm VF1 film was used with 12 ml/min helium column flow rate, 20 °C/min temperature programming rate was used and the elution times and temperatures are indicated. One microliter carbamate pesticide mixture in acetonitrile was used (531.1 LC mixture from Restek, Bellefonte, PA, USA) with the 1079 injector having split ratio of 20:1 at 150 °C injector temperature. Below the chromatogram, representative mass spectra are shown including NIST library EI MS (upper MS trace), cold EI (second trace), methanol cluster CI (third trace) and methanol cluster CI with methanol reservoir heated to 40 °C (bottom trace) for aldicarb (left) and oxamyl (right).

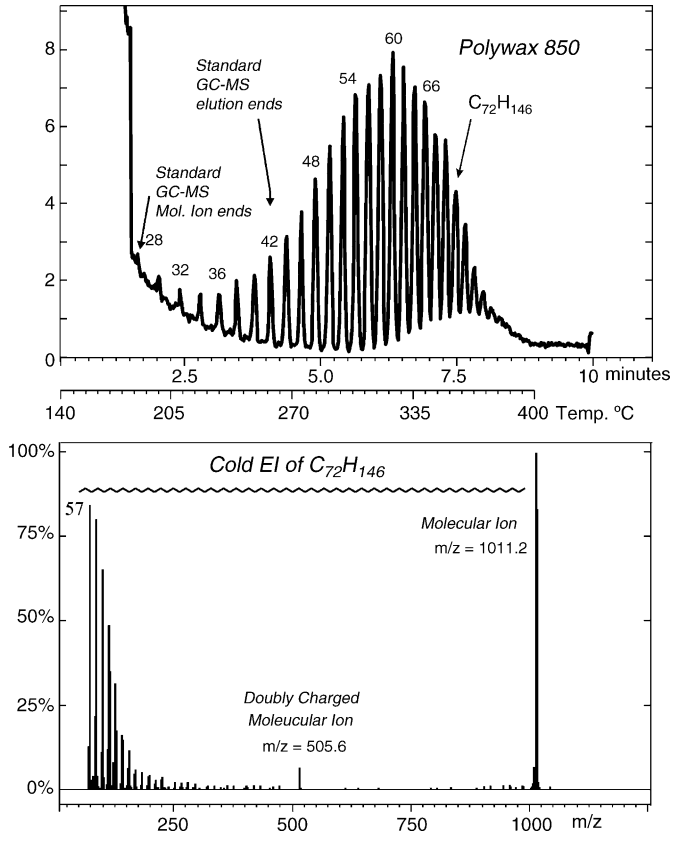

Fig. 4. The analysis of large aliphatic hydrocarbons with the 1200-SMB. Full scan mass chromatogram is shown in the upper trace with carbon numbers of a few compounds. The cold EI mass spectrum of $C_{72}H_{146}$ is shown in the bottom mass spectrum. Polywax 850 sample (Restek, Bellefonte, PA, USA) was diluted at about 0.2% in xylene and injected into a 1079 injector at 250 °C injector temperature, which was ramped in 1 min to 400 °C. Four meters column with 0.1 μm VF1 film was used with 32 ml/min helium column flow rate and the GC oven was temperature programmed to 400 °C at 30 °C/min.

the 1200-SMB system has upper mass range of 1500 amu we decided to explore the analysis of much larger hydrocarbons, both in terms of their elution from the GC column and mostly in terms of their mass spectra and availability of molecular ions. Since all large hydrocarbons show the same C_nH_{2n+1} fragment ions, without having a molecular ion GC–MS analysis is void of useful information and in that case it is even inferior to GC-FID. Thus, having a molecular ion is crucial for the GC–MS analysis of large hydrocarbons.

In Fig. 4, the analysis of large hydrocarbons with the 1200-SMB system is demonstrated. We used a Polywax 850 (Restek, Bellefonte, PA, USA) mixture of large linear chain hydrocarbons with even number of carbon atoms. A 4 m Varian VF1 column with 0.1 μm film and upper temperature limit of 400 °C was used with 32 ml/min helium column flow rate and 30 °C/min GC oven temperature programming rate.

Under these conditions, despite the reduced GC separation resolution all the hydrocarbons were properly separated. The most important observation shown in Fig. 4 is that a dominant molecular ion is obtained, even for a milestone $C_{72}H_{146}$ hydrocarbon that has a molecular ion above 1000 amu (1011.2 amu). We note that the nominal mass of this compound is 1010 but due to the mass defect of 146 hydrogen atoms its real mass is shifted by 1.2 amu. In addition, we observed dominant molecular ions all the way to $C_{84}H_{170}$ of 1197.3 amu. To the best of our knowledge these are the largest hydrocarbons that were analyzed by GC–MS with molecular ions. In fact, GC–MS analysis with standard 30 m column with 1 ml/min column flow rate ends at the analysis of about $C_{40}H_{82}$ or up to $C_{44}H_{90}$. Furthermore, already above $C_{28}H_{58}$ the molecular ion is practically missing. Thus, in Fig. 4 we demonstrate the doubling of the range of hydrocarbons that can be analyzed by GC–MS and tripling the range of hydrocarbons that show a molecular ion hence that their analysis can be considered as useful and informative. We note that the problems of weak or no molecular ions are further exacerbated in standard GC–MS analysis since in order to prevent ion source peak tailing with the low volatility hydrocarbons the ion source must be heated to high temperatures which result in an exponential reduction of the relative abundances of the molecular ions [24,25]. With the 1200-SMB fly-through cold EI ion source no ion source peak tailing is ever observed and such tailing can originate only from the transfer line if it has a cold spot. Thus, for obtaining Fig. 4 we carefully eliminated any transfer line cold spot, it was maintained at 400 °C and the nozzle was at 360 °C (with 90 ml/min He flow rate).

The observation of dominant molecular ions for these large hydrocarbons is not a trivial matter. It is important to realize that as the sample compounds become bigger its vibrational cooling quickly becomes ineffective. The reason for this is that prior to any vibrational cooling the sample must be accelerated to the helium velocity and overcome the velocity slip effect [26]. As the hydrocarbon becomes bigger it must be proportionally accelerated to higher kinetic energies while its acceleration by each collision is lower since the mass ratio with helium is larger. Thus, the number of helium collisions required to accelerate the sample grow at the second power of the sample compound molecular weight. After acceleration, the vibrational cooling efficiency per collision also becomes less effective since the sample heat capacity increases with the sample size and the nozzle temperature must be increased with the reduced sample volatility. On the other hand, electron ionization of hydrocarbons is a localized process that does not depend on the compound carbon chain length. Thus, the excess vibrational energy produced by electron ionization is hydrocarbon size independent and after vibrational cooling it is redistributed among increased number of vibrational degrees of freedom. Thus, if the hydrocarbons are properly vibrationally cooled, then we should expect to see only a molecular ion for these large hydrocarbons. Consequently, we believe that the experimentally observed cold EI mass spectra are the result of a compromise between two conflicting trends with increased carbon chain length: (a) incomplete vibrational cooling and (b) increased vibrational heat capacity, and the net result is the observed dominant molecular ion combined with the usual low mass fragments.

As in any new unexplored "territory" we found an unexpected result of doubly charged molecular ions peak of $m/z = 505.6$, which is shown in Fig. 4 at the bottom mass spectrum. We found the onset of appearance of these peculiar ions to be around $C_{46}H_{94}$ and their relative abundance steadily grows with

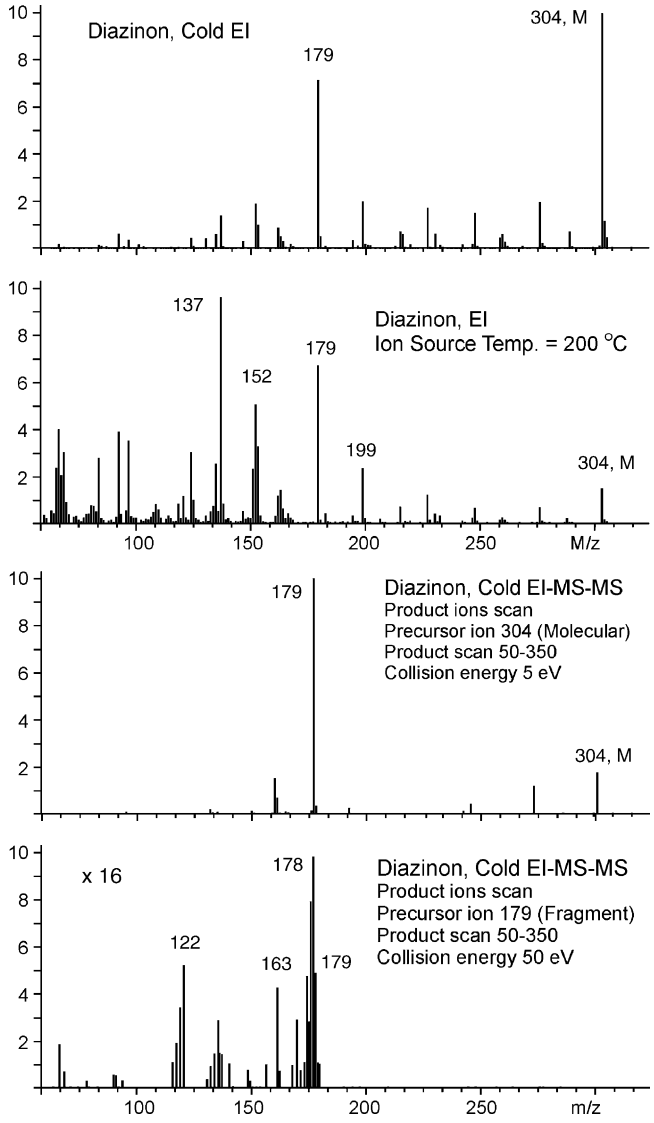

Fig. 5. EI mass spectra and MS–MS of diazinon ($C_{12}H_{21}N_2O_3PS$). Cold EI mass spectrum of diazinon (upper MS trace) is compared with its standard EI mass spectrum obtained at 200 °C ion source temperature. MS–MS of diazinon on the molecular ion ($m/z = 304$, third trace) is compared with MS–MS of diazinon on its $m/z = 179$ fragment (bottom trace).

the hydrocarbon chain size above that onset. Since these ions are clearly less stable than the molecular ion due to intra ion Columbic repulsion forces, the vibrational cooling is essential for their existence. The subject of doubly charged ions is beyond the scope of this paper and will be discussed in a future publication [27].

3.4. Mass spectrometry mass spectrometry (MS–MS) with SMB

The central feature of the Varian 1200 system is its effective triple quadrupole based MS–MS capability. Thus, an important part of this research was to expose the benefits of cold EI for MS–MS.

In Fig. 5, the cold EI mass spectrum of diazinon (upper trace) is compared with that obtained with the same 1200 system with its standard EI ion source maintained at 200 °C. It can be seen that the molecular ion relative abundance is enhanced by approximately an order of magnitude. In Fig. 5 third trace, we show the MS–MS daughter ions mass spectrum obtained with 5 eV collision energy with the CAD argon gas in Q_2, while the precursor ion is the molecular ion 304 amu produced by cold EI. A dominant $m/z = 179$ fragment ion appears, which is very useful for analytical MS–MS purposes. On the other hand, when we tried to dissociate the $m/z = 179$ fragment ion with 5 eV collision energy it did not dissociate and 50 eV collision energy was required to obtain the MS–MS spectrum shown at the bottom trace in Fig. 5. Thus, MS–MS on the fragment was both inefficient as well as much less sensitive as demonstrated by the 16 times gain used in Fig. 5 bottom trace.

The above observation can be rationalized by the fact that the molecular ion is often unstable hence can be easily dissociated to a given dominant fragment. However, this fragment by its definition as dominant must be a stable fragment ion hence its further dissociation could be harder. Once we need to use high collision energy than many channels are opened and the sensitivity is sacrificed. Thus, MS–MS on the molecular ion was found to be more sensitive than on other fragment ions and the cold EI enhancement of the molecular ion enters directly into enhanced MS–MS sensitivity. In addition, as is well known [7] the molecular ion suffers from much lower amount of matrix interference and thus MS–MS on the molecular ion is also more selective than MS–MS on a fragment.

3.5. Fast GC–MS–MS analysis with the 1200-SMB

The subject of fast GC–MS was recently the focus of many studies and a central topic of research with the Supersonic GC–MS. We believe that effective fast GC–MS requires the optimization of several parameters and we feel that the fast scan speed of time-of-flight mass analyzers or fast temperature programming rate are not as important as commonly believed. However, we prefer to avoid a controversial discussion of this subject as we did in the past [5]. We consider the system's selectivity as the most important parameter for fast GC–MS [5] since with every factor of two increased MS selectivity and its separation power one can reduce the GC separation power by a factor of two and gain a factor of four faster analysis time without trading sensitivity and other central analytical parameters. For example, with four times shorter column the GC separation is reduced by a factor of two while the analysis can be four times faster with four times faster temperature programming rate while keeping the elution temperatures practically unaffected. In addition, the use of a very high column flow rate with the 1200-SMB facilitates fast splitless injections without affecting the sensitivity, at high GC oven temperatures. The reason for this is that if the splitless injection is fast, than column cryo-focusing is not needed and the injection can be performed at the analytically useful sample elution temperature. This way one can save 1 min splitless injection time, 1–2 min temperature programming up to the sample elution temperature and 2–3 min for cooling down to, say, 50 °C that is needed for the cryo-focusing. Thus, high flow rate based fast splitless injection saves typically 5 min from the

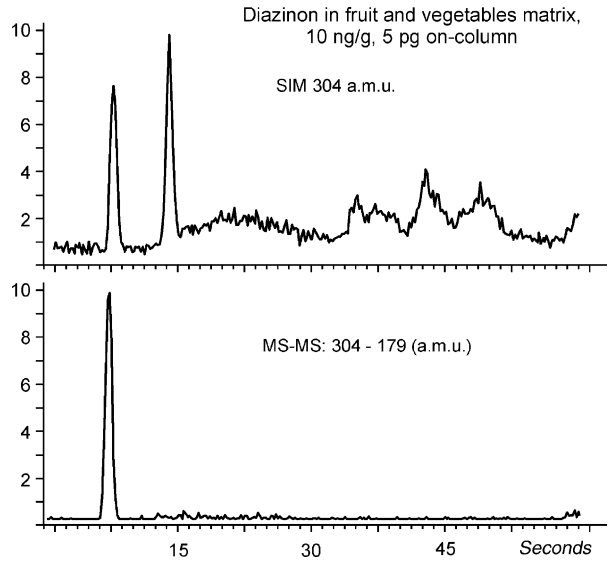

Fig. 6. Fast GC–MS and GC–MS–MS analysis of diazinon at 10 ng/g concentration in a representative eight fruit and vegetable extract with the 1200-SMB. Four meters column with 0.1 μm VF1 film was used with 32 ml/min helium column flow rate. The GC oven initial temperature was 130 °C and it was temperature programmed at 50 °C/min for 1.4 min to 200 °C. 1079 injector was used at 250 °C with 1:1 split ratio so that 5 pg diazinon were injected into the column. The upper trace is the SIM chromatogram obtained on the molecular ion at $m/z = 304$ while the bottom trace was obtained with MS–MS (304–179 amu) using the conditions of Fig. 5 third trace.

analysis, which is critical for any effective fast GC–MS analysis. In Fig. 6, we demonstrate a few seconds long GC–MS–MS analysis of diazinon, spiked at 10 ng/g in a mixed fruit and vegetable extract (courtesy of S. Lehotay and K. Mastovska of the USDA). Note that diazinon elutes after 7 s. The upper trace was obtained in the SIM mode on the molecular ion $m/z = 304$ while the bottom trace was obtained using MS–MS on the molecular ion and $m/z = 179$ daughter ion. A clean MS–MS trace is obtained even for this 10 ng/g "trace" level analysis in complex matrix. We used 32 ml/min helium column flow rate and split ratio of 1:1 so that 5 pg dizinon were analyzed, yet the extrapolated detection limit is approximately 0.1 ng/g (50 fg). While diazinon eluted after 7 s using injection at 130 °C, we used 50 °C/min temperature programming rate and cooling down after 1.2 min thus repetitive analysis could be obtained after each 3 min. Probably we could use isothermal injections and employ temperature programming only after every 10 or more analyses for cleaning the column but we have not investigated this opportunity at this time. The important observation that is demonstrated in Fig. 6 is that the combination of enhanced selectivity in using both enhanced molecular ion in cold EI and MS–MS on the molecular ion enables the suppression of matrix chemical noise even under very fast GC–MS analysis and it is the key for such fast analyses.

3.6. Sensitivity of the 1200-SMB system

Sensitivity is considered as one of the most important specifications of any GC–MS. However, the sensitivity of GC–MS is often poorly defined and often users are surprised that they are unable to obtain or even approach the specified sensitivity with their own samples. The main reason for this is that GC–MS sensitivity is usually specified with octafluoronaphthalene (OFN) which is a highly inert, relatively volatile compound whose mass spectrum is dominated by a molecular ion that amounts to over 30% of all its ions and that has no major isotopomers. In addition, the OFN molecular ion is at $m/z = 272$ that has the lowest amount of vacuum background in clean vacuum chambers in the 100–300 amu mass range. On the other hand, GC–MS is typically used with more difficult compounds that often have either weak or no molecular ion and with vacuum chambers that show all the history of samples that were injected hence with pronounced vacuum background, particularly when complex matrices are analyzed. Furthermore, some say that the analysis is as good as its weakest samples thus, sensitivity is important particularly for those samples that are hard to analyze.

In Fig. 7, the sensitivity of the 1200-SMB system is shown with both OFN and decabromobiphenylether (DBBPE). The upper trace shows the single ion mass chromatogram obtained with the injection of ~1 μl, 1 ng/ml OFN sample with split ratio of 5 into a 4 m column with 4 ml/min helium column flow rate. The 200 fg on-column OFN gave signal to noise ratio of over 7000 in RMS units and about 320 in peak-to-peak units. Using ion counting we found that the signal is 12 ions per femtogram while the noise was about 3 ions/s, which is less than one ion per 200 ms dwell time that was used. In the insert to Fig. 7 upper trace one can see the noise magnified 50 times. The OFN signal level was similar to that obtained with the standard 1200L GC–MS ion source but the noise level was lower and thus we could demonstrate crossing down the 1 fg LOD milestone. In the 1200-SMB, the origins of noise are completely different than with standard GC–MS. The noise in the standard 1200 system is fully due to vacuum background and depends on the cleanliness of the vacuum chamber and its history. With cold EI, vacuum background is effectively filtered due to the zero electric field of the dual cage ion source and different ion kinetic energies of forward moving sample ions in the SMB compared with random moving low kinetic energy vacuum background ion, as explained in Refs. [10,11,22]. Thus, the main noise source in the 1200-SMB was found to be the cleanliness of the make up gas transfer line and, particularly, heated transfer line. When the transfer line was cooled to 100 °C we could obtain less than eight ions per 500 ms scan in the 50–700 amu range and 3 ions/s at $m/z = 272$ as demonstrated in Fig. 7 (upper trace insert). With the transfer line at 260 °C the full scan noise was increased three times to 24 ions/scan but since OFN eluted at 65 °C in our system we could use 100 °C transfer line temperature. Due to this low mass spectral noise we also obtained for 1 pg OFN a "noise free" full scan RSIM mass chromatogram (50–300 amu MS range) that had no single ion noise thus there is no point in showing it in this manuscript.

In comparison with other GC–MS's our OFN signal is similar to that of standard systems and noise is lower, thus we can obtain a little lower detection limit as demonstrated in Fig. 7. However, we feel that the 1200-SMB is characterized by superior sensitivity for the more difficult samples and the harder is

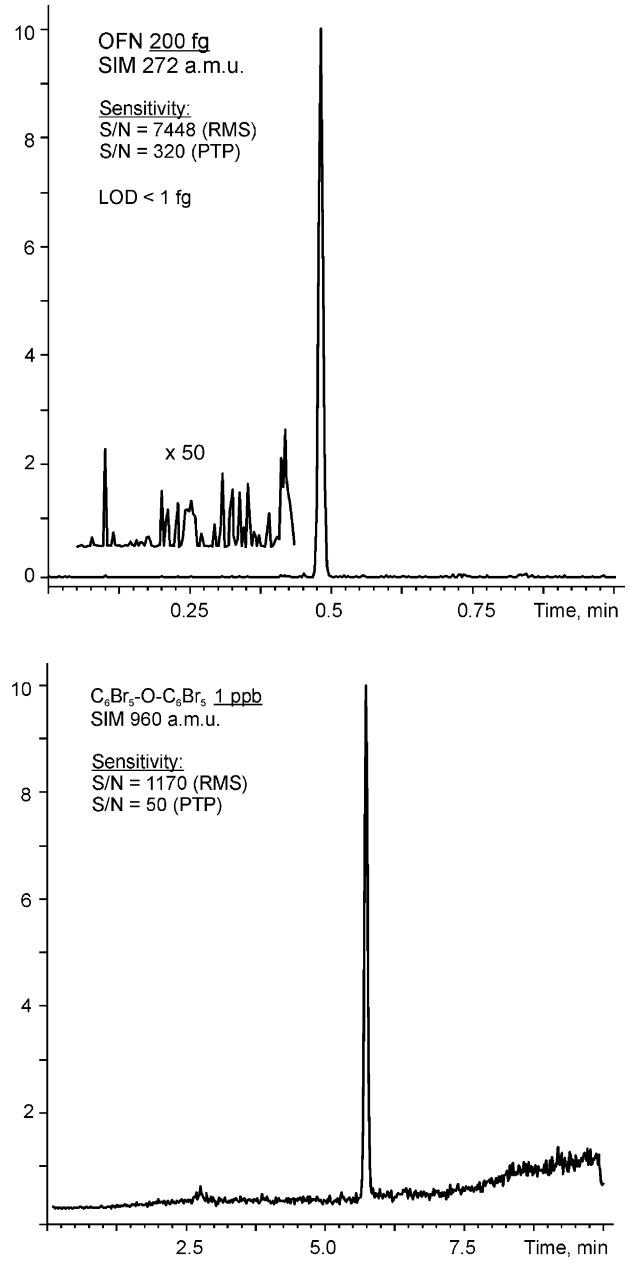

Fig. 7. Sensitivity evaluation of the 1200-SMB system. The upper trace was obtained with OFN sample of 1 ng/ml with ~1 μl injected using a 1177 injector with split ratio of 5. Four meter VF-Xms column was used with 4 ml/min He column flow rate. The transfer line temperature was 100 °C and the initial GC oven temperature was 50 °C with programming rate of 30 °C/min. SIM at $m/z = 272$ was used. The bottom trace was obtained with 1 ng/ml sample of decabromobiphenylether. SIM at $m/z = 960$ was employed and the 1200 resolution was 6 amu. Four meter column with 0.1 μm VF1 film was used with 16 ml/min helium column flow rate. The GC oven initial temperature was 110 °C and it was temperature programmed at 20 °C/min. Two microliter was injected splitless.

the compound analysis by standard GC–MS the greater is the gain in 1200-SMB sensitivity.

In Fig. 7 lower trace we demonstrate the sensitivity of the 1200-SMB system for decabromobiphenylether. This compound has a nominal molecular weight of 960 amu and it is considered to be somewhat thermally labile and difficult for analysis. In Fig. 7, we show that with the splitless injection of 1 ng/ml sample (2 μl) in SIM mode we obtain S/N = 1170 RMS or ~50 in peak-to-peak. Considering the 10 times broader DBBPE GC peak the demonstrated sensitivity is not too far from that of OFN. In order to reduce high mass quadrupole transmission and isotope distribution losses (10 bromine containing molecular ion with 25 isotopomer peaks, 7 of which are significant) we used low, 6 amu, mass resolution, but for this high mass compound this is acceptable since no other known compound is expected to coelute with it. The noise in the DBBPE chromatogram is mostly due to injector memory effects of past DBBPE injections of higher concentration samples and it can be further reduced.

As shown in Fig. 7 the OFN SIM sensitivity of 1200-SMB is slightly superior to that of the original 1200 GC–MS. However, for more difficult samples and, particularly, for those that have no or only weak molecular ions the enhancement of the relative abundance of the molecular ion serves for improved sensitivity of the 1200-SMB as shown in Fig. 8.

Cholesterol can be considered as a typical sample that is a relatively late eluter and, although, its mass spectrum shows a molecular ion, the mass spectrum is very complex, with many fragments and the share of the molecular ion is thus weak. The cold EI mass spectrum of cholesterol was investigated in the past and it is characterized by significant enhancement of the molecular ion [28]. In Fig. 8 (upper trace), the full scan RSIM chromatogram of cholesterol is shown and compared with that obtained using 4 m column and 8 ml/min helium column flow rate with the 1200-SMB. Over 50 times increased signal to noise ratio was obtained with the 1200-SMB for cholesterol. Not surprising is the fact that the 10 ng RSIM trace of n-$C_{32}H_{66}$ was void of signal and it showed only noise while a clean baseline was obtained with the 1200-SMB, demonstrating over 2000 times sensitivity enhancement with cold EI. We note that for hydrocarbons the molecular ion is the only characteristic ion thus this is a real gain in sensitivity for these compounds.

3.7. Linear dynamic range and reproducibility

Linear dynamic range and reproducibility are important features of any GC–MS. Currently the 1200-SMB is under development and its ionizing electron emission current is not properly regulated, although appropriate electronics is under development. As a result, we have only preliminary linearity and reproducibility data. We tested the system linearity with pyrene samples using SIM at its molecular ion $m/z = 202$. We injected splitless on-column samples of 1 pg, 10 pg, 100 pg, 1 ng and 10 ng, and obtained linear calibration curve with $R^2 = 0.9988$. Thus, the system is linear over full four orders of magnitude and probably the linear dynamic range (LDR) extends beyond this range. Somewhere above 10 ng on-column pyrene, saturation of the electronics is encountered. We decided not to test an additional point with 0.1 pg pyrene in view of minor injector carry-over effects of pyrene residue from past experiments with the 1177 GC injector. Reproducibility seemed to be reasonably good, for example, it was about 4–5% R.S.D. in the analysis of testosterone in alligator blood extracts. We are aware of the

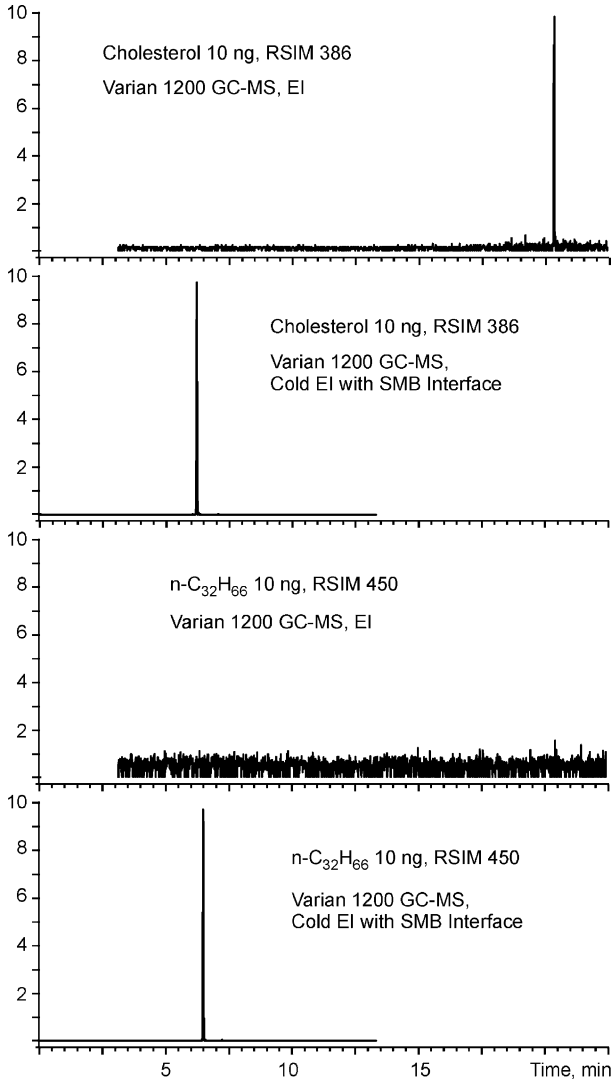

Fig. 8. Sensitivity evaluation of the 1200-SMB with cholesterol and $C_{32}H_{66}$. Ten parts per million samples were injected in the full scan mode. The upper and third traces were obtained with the 1200 system before its conversion into 1200-SMB using standard 30 m VF5MS column and 1 ml/min He flow rate. RSIM on the molecular ions $m/z = 386$ and 450 are shown. The second and bottom traces were obtained for these compounds with the 1200-SMB using 4 m column with 0.1 μm VF-Xms film and 16 ml/min He column flow rate.

need to better characterize the system reproducibility, and plan to work on it after having appropriately improved electronics.

4. Discussion and conclusions

A new GC–MS instrument was designed built and evaluated, based on the combination of the Varian 1200L tripe quadrupole GC–MS with a supersonic molecular beam interface and its related fly-through electron ionization ion source for the ionization of vibrationally cold sample molecules while contained in the SMB. We named this system as 1200-SMB.

The 1200-SMB provides "cold EI" mass spectra with enhanced molecular ion and mass spectral information which improves the confidence level in sample identification and NIST library identification probability. The feature of enhanced molecular ion also increases the system sensitivity via SIM, RSIM and/or MS–MS on the molecular ion.

Fast analysis including of trace levels in complex matrices is enabled via fast splitless injection at high GC oven temperatures and the increased matrix interferences associated with the shorter analysis time are suppressed through the increased systems selectivity due to the enhanced selectivity of both the molecular ion and MS–MS. The use of high column flow rate for lowering the sample elution temperature for the GC column (and injector liner) and total elimination of any ion source degradation enables the analysis of significantly increased range of thermally labile and low volatility samples. This important feature was demonstrated with carbamate pesticides and large aliphatic hydrocarbons all the way to $C_{84}H_{190}$, combined with the provision of dominant molecular ions to all these large compounds. While the 1200-SMB signal is similar for simple compounds such as OFN its sensitivity is increased for harder to analyze compounds due to three reasons of (a) enhanced molecular ion; (b) elimination of ion source peak tailing and (c) elimination of vacuum background. When these three reasons are combined, remarkable sensitivity enhancement factors exceeding 100 up to qualitative differences can be obtained. The experimental system is under continuous development and improvements. One major additional near future feature will be the incorporation of automated isotope abundance analysis (IAA) software [29] which utilizes the feature of enhanced molecular ion to obtain elemental formulas from its measured isotope abundances using unit mass resolution quadrupole mass analyzer.

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